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PRINCIPAL INVESTIGATOR: Stephen Baylin, Ph.D.

CONTRACTING ORGANIZATION: Johns Hopkins University School of Medicine
Baltimore, MD 21205

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Stephen Baylin, Ph.D.

E-mail: sbaylin@jhmi.edu

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14. ABSTRACT

Fifteen to 20% of women with initial DCIS subsequently manifest invasive breast carcinoma. At present, there is no real means to predict which patients will undergo this disease progression and recurrence. The purpose of this IDEA, DOD award is to determine whether hypermethylation of the *AP-2*α, and other genes, may identify those DCIS lesions at high risk for recurrence as invasive breast carcinoma. The idea was based on a small initial study (~ 15 samples) where we found only ~20% of DCIS samples, versus 70% of invasive tumors, had *AP-2*α hypermethylation. Our first chief aim was to verify these findings and identify other genes which might also prove useful as prognostic markers. During the past year, we have accomplished this goal by deriving a panel of 5 genes, including *AP-2*α, any two of which when hypermethylated in DCIS, preliminarily tracks with patients who develop recurrent disease. Each marker, in the main, has a much higher incidence of hyper-methylation in invasive breast cancer versus DCIS. We are now ready, in the definitive case control study with UCSF, to test this panel of genes for its efficacy in predicting cases of DCIS which are likely to recur. We have also used the panel in a study with Steve Belinsky and are awaiting breaking of the code for longitudinal five year follow up to see how the results track with recurrent disease.

15. SUBJECT TERMS
Breast Cancer, DCIS, *AP-2*α, Hypermethylation, SOCSI, *E-cad* genes

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Table of Contents

Introduction

The overall purpose of this Idea Award remains exactly as stated in the originally funded proposal. In this regard, ***the overall study objective remains to determine whether hypermethylation of the AP-2α promoter, and of other promoters where the marker incidence looks promising, could serve as molecular markers to predict the potential for breast DCIS (ductal carcinoma in-situ) lesions from individual patients to recur as invasive breast cancer***. In-situ carcinomas account for approximately 20% of all newly diagnosed breast cancers in women. So the question of predicting which of these lesions which may progress, following initial surgery, to invasive breast cancers is an extremely important one especially since this may occur in some 15 to 20% of women diagnosed with DCIS. For our objective, we are employing a newly established, and highly sensitive, PCR assay for hypermethylation of *AP-2α* exon1, and for other candidate genes, which can be utilized on DNA scraped from slides prepared from paraffin blocks. In carrying out our overall objective, our study has three specific aims which have remained totally unchanged from the original proposal and these are:

Task/Specific Aim #1 – To verify the low incidence of *AP-2α* exon 1 hypermethylation in pure DCIS and compare this incidence in a progression model that assesses methylation in DCIS adjacent to and in the invasive cancer.

Task/Specific Aim #2 – To conduct a nested case control study, in collaboration with the UCSF Breast Cancer SPORE group, of *AP-2α* hypermethylation in DCIS samples from patients who have and have not manifested recurrent invasive disease.

Task/Specific Aim #3 – To conduct a prospective study of the predictive power for *AP-2α* hypermethylation in a cohort of women from New Mexico with DCIS.

Body

During the past year, we have made our most significant progress for this proposal and taken a next step towards the ultimate goals of this work. We now have a panel of genes which, collectively, appear to hold promise for predicting the behavior of DCIS. The results now position to receive the most valuable longitudinal samples from UCSF to validate the promise of our approach.

Specific Aim #1: To verify the low incidence of *AP-2α* exon 1 hypermethylation in pure DCIS and compare this incidence in a progression model that assesses methylation in DCIS adjacent to and in the invasive cancer. At the time of last year's progress report, we had studied 47 new samples of known DCIS, without longitudinal follow-up and concluded that hypermethylation of *AP-2α* is seen in 9 of 47 or 19%. We have now also developed a panel of an additional four genes for study of hypermethylation DCIS samples. For the AP-2α methylation status and that of these other genes, we have now looked a set of DCIS cases supplied by our collaborators at UCSF consisting of 10 patients whose tumors recurred and 10 whose did not. These have been matched for date of diagnosis.

For the 5 gene panel, consisting of *Ap2, Cyclin D, E-cadherin, Socs-1* and *GSTpi,* the data shows that the odds of having recurred versus not recurred tumors is 5.98 times higher for those with a methylated gene panel compared to those without (95% CI:0.81, 35.5, p=0.08 – **Figure 1**). A methylated gene panel is being defined as any sample having greater than 2 genes being methylated. The wide confidence interval is secondary to the small sample size, which will improve with increased numbers of samples.

		UCSF CODE	CYCLIN D	GSTP	ECAD	SSOCS	AP2	Total genes M
1	Recurred	2440F	0	0	0	0	0	0
2	Recurred	6373D	0	1	0	1	0	2
3	Recurred	89-S-1839 2E	0	1	0	1	0	2
4	Recurred	13869B	0		0	0	0	0
5	Recurred	F45-1E	1	0	0	0	1	2
6	Recurred	F23-2K	0	0	0	0	0	0
7	Recurred	5186F	1	0	0	1	0	2
8	Recurred	C94-10082B	1	1	0	1	0	3
9	Recurred	8474A	0	0	0	0	0	0
10	Recurred	6480B	1	1	0	1	0	3
11	Non-recurred	203B	1	0	0	0	0	1
12	Non-recurred	S621	0	0	0	1	0	1
13	Non-recurred	167A	0	0	0	0	0	0
14	Non-recurred	2114C	0	0	0	0	0	0
15	Non-recurred	S91-3403 A5	0	0			0	0
16	Non-recurred	271D	0	1	0	0	0	1
17	Non-recurred	584G	0	1	0	0	0	1
18	Non-recurred	1092B	0	0	0	0	0	0
19	Non-recurred	D-135C	1	0	0	1	0	2
20	Non-recurred	6838A	1	1	0	1	0	3

Fig. 1 – Methylation status of a 5 gene panel in DCIS tumors from patients whose disease which did or did not recur over a five year period. *Green = non-methylated promoter; Red = methylated promoter. The total genes hypermethylated from the panel in a given tumor is provided in the last column. As shown only 2 of 10 non-recurrent tumors had two or more genes hypermethylated while 6 of 10 tumors that recurred had this change. Note that for AP2α, hypermethylation occurred only in a tumor that recurred.*

In these 20 samples, 6 of the samples from DCIS lesions that recurred have greater than 2 genes methylated. In the DCIS samples of patients who have not recurred, 2 of the samples have greater than 2 genes methylated. With a Fischer's exact evaluation, this difference is not statistically significant (2 tail, p=0.169). However, if the sample size was doubled, this would have been statistically significant (2 tail, p=0.02).

Additionally, we have analyzed the genes on an additional sample set for which the code has not yet been broken for disease recurrence and this group contains 10 High Grade DCIS samples and 10 Low Grade DCIS samples. We looked at the data for this same multiplicity with the gene panel. Data shows that in the HGDCIS lesions, we had 3 out of 10 samples showing greater than 2 genes methylated and in the LG-DCIS lesions, there were 0 out of 10 samples that had greater than 2 genes methylated.

The above methylation pattern is consistent with the prediction that the methylation results may reflect the biological pattern expected for progression of DCIS to a more aggressive phenotype. The lesions which are recurring might have a methylation profile which is more like the invasive breast cancer lesions, which frequently have multiple genes methylated. To look at this question we have studied our panel (Figure 2) for direct comparison of each gene's methylation percentages in 73 cases of invasive breast cancer, DCIS associated with invasive breast cancer, high grade DCIS, low grade DCIS, samples of DCIS where the patient has recurrent disease, and samples of DCIS where the patient has no recurrence. It can be seen from the above data that the incidence of hypermethylation for virtually all of the genes is distinctly higher in invasive tumors than in the total samples of DCIS and that the results for the group of DCIS samples which recurred (next to last panel in the graph), the incidence for the genes is more similar to those for the invasive tumors.

	N	AP2	CYCLIND	GSTP	ECAD	SSOCS
Breast Tumors	73	55%	40%	47%	29%	55%

	N	AP2	CYCLIND	GSTP	ECAD	SSOCS
IBC	7	14.30%	42.90%	28.60%	14.30%	71.40%
DCIS associated w IBC	7	0	42.50%	14.20%	14.20%	28.50%
HGDCIS	10	10%	20%	30%	0	30%
LGDCIS	10	0	10%	10%	10%	12.50%
Combined HGDCIS_LGDCIS	20	5%	15%	20%	5%	20%

	N	AP2	CYCLIND	GSTP	ECAD	SSOCS
Recurred DCIS	10	10%	40%	44%	0%	50%
Not Recurred DCIS	10	0%	30%	30%	0%	30%

Figure Two: Percent methylation of each gene in samples of invasive breast cancer and DCIS.
Methylation results shown for invasive breast cancers, ductal carinoma in situ, high grade DCIS, low grade DCIS, recurrent and non-recurrent DCIS

Specific Aim #2: To conduct a nested case control study, in collaboration with the UCSF hypermethylation in DCIS samples from Breast Cancer SPORE group, of AP-2 patients who have and have not manifested recurrent invasive disease. Given the above data, we are excited about its implications and utility as a molecular marker to identify patients with recurrent DCIS. We are now arranging to pursue the most critical evaluation of this proposal, examination of the additional samples for a nested case control study from UCSF. We have performed the power calculations to see significant predictive results for our panel and this suggests that an additional 47 samples of each recurrent and non-recurrent DCIS lesions (total of 94 samples) would yield 90% power (p0=0.2, p1=0.5). It is our understanding from our collaborator, Joe Gray at UCSF, that we may especially obtain these samples from DCIS lesions where four or more slides are available.

Specific Aim #3 – To conduct a prospective study of the predictive power for *AP-2α* hypermethylation in a cohort of women from New Mexico with DCIS. This is the other ultimate study in the proposal utilizing the cohort from Dr. Belinsky in which DCIS samples, over 100, come from a 3,000 patient study of women with early stage breast cancer and/or DCIS in New Mexico. These patients have complete 5 year longitudinal follow-up and this has just been successfully complied into a finalized database. We now have actually studied all of the samples from all of the women with DCIS that have been identified. In addition to examining the methylation status of *AP-2α* , we have expanded the work to look at each of the other candidate genes in our panel discussed in detail in Specific Aim #1. The longitudinal data for recurrence are now in progress by Dr. Belinsky and his colleagues and we will match the results to our findings as soon as these are available.

Key Research Accomplishments

As outlined above, we have made significant strides during the past year and are excited to complete our studies during the coming year by performing the nested case control study for DCIS recurrence which is the ultimate goal of our work. We anticipate that this study will give us the initial answers whether our hypermethylation markers have predictive behavior for the behavior for DCIS.

Reportable Outcomes

The initial results upon which the Idea Award was based are now published Douglas DB, Akiyama Y, Esteller, M, Gabrielson E, Weitzman S, Williams T, Herman JG, Baylin SB. Hypermethylation of a Small CpGuaine-Rich Region Correlated with Loss of Activator Protein-2α Expression during Progression of Breast Cancer. 64:1611-1620, 2004.

Conclusions

All final conclusions must await the studies outlined for Specific Aim #2 and 3, as outlined above.

References

No papers have yet been submitted from the work done directly with support of this award.

Appendixes

None